

LoopSeq amplification conditions

Full-length 16S and 18S+ITS sequences underwent several amplification procedures, prior to the reconstruction of the LoopSeq synthetic long reads: enrichment, barcoding, and universal amplification. To ensure the amplification of full-length molecules, samples were initially amplified using standard primers. The 16S sequences samples were subjected to 22 cycles of amplification using 27F/1429R primers (<https://doi.org/10.1128/jb.173.2.697-703.1991>), while 18S+ITS used 27 cycles of amplification with the SR1-R primer specific to 18S and a mix of primers for the ITS region represented in several fungal species: LR1, LR15, ITS4_KYO1 and ITS4_KYO3 (https://sites.duke.edu/vilgalyslab/rdna_primers_for_fungi/, <https://doi.org/10.1128/jb.172.8.4238-4246.1990>, & <https://doi.org/10.1371/journal.pone.0040863>). The amplicon products were diluted 1:100 and used as input into the LoopSeq assays. The samples underwent 5 - 10 cycles of amplification with oligos that contained the gene specific regions as well as LoopSeq specific binding sites. The LoopSeq enriched libraries were treated to 10 cycles of amplification to add both unique molecular indexes as well as positional barcodes to all molecules within a given sample. Barcoded samples were then diluted and normalized to subsample ~10,000-full length molecules per sample. These normalized libraries were then amplified for 22 cycles using universal LoopSeq adapter primers to create multiple copies of the UMI-tagged molecules. All libraries were pooled by volume, prior to being processed into standard short read libraries for PE150 bp sequencing on the AVITI platform.

Initial PCR - 16S (27F/1429R Primers)

95.0 C for 3:00

22 cycles:

- 95.0 C for 30 sec
- 52.0 C for 45 sec
- 72.0 C for 2 min

10.0 C Hold

Initial PCR - 18S+ITS (SR1-R, LR15, ITS4_KYO1, ITS4_KYO3 Primers)

95.0 C for 3:00

27 cycles:

- 95.0 C for 30 sec
- 52.0 C for 45 sec
- 72.0 C for 2 min

10.0 C Hold

Enrichment PCR - 16S+LoopSeq Handle

95.0 C for 3:00

10 cycles:

- 95.0 C for 30 sec
- 52.0 C for 45 sec
- 72.0 C for 2 min

10.0 C Hold

Enrichment PCR - 18S+ITS+LoopSeq Handle

95.0 C for 3:00

5 cycles:

- 95.0 C for 30 sec
- 52.0 C for 45 sec
- 72.0 C for 2 min

10.0 C Hold

Barcoding PCR - 16S

95.0 C for 3:00

10 cycles:

- 95.0 C for 30 sec
- 65.0 C for 45 sec
- 72.0 C for 2 min

10.0 C Hold

Barcoding PCR - 18S+ITS

95.0 C for 3:00

10 cycles:

- 95.0 C for 30 sec
- 65.0 C for 45 sec
- 72.0 C for 4 min

10.0 C Hold

Universal PCR - 16S

95.0 C for 3:00

22 cycles:

- 95.0 C for 30 sec
- 60.0 C for 45 sec
- 72.0 C for 2 min

10.0 C Hold

Universal PCR - 18S + ITS

95.0 C for 3:00

22 cycles:

- 95.0 C for 30 sec
- 60.0 C for 45 sec
- 72.0 C for 4 min

10.0 C Hold